AMELIORATION OF LIPIDS BY *EUGENIA CARYOPHYLLUS* EXTRACT IN ATHEROGENIC DIET INDUCED HYPERLIPIDEMIA

ROMA GHAI¹, KANDASAMY NAGARAJAN², VINAY KUMAR³, MINU KESHERI⁴, SWARNA KANCHAN⁵

ABSTRACT

The present study focus on the lipid lowering potential of aqueous ethanolic extract of *Eugenia caryophyllus* in high fat diet induced hyperlipidemia. Hyperlipidemia was induced in rats by oral administration of high fat diet for six weeks. The extract was thereafter administered at two different doses of 200mg/kg and 400mg/kg body weight for next six weeks to hyperlipidemic rats. Atorvastatin was used as a reference standard. The extract exhibited anti-hyperlipidemic activity in dose dependent manner. The dose of 400mg/kg exhibited maximum antihyperlipidemic efficacy with values of total cholesterol as 145.84±1.11mg/dl, LDL-C as 87.85±1.75mg/dl, VLDL-C as 28.52±0.30mg/dl, HDL-C as 29.47±1.28mg/dl, atherogenic index as 4.99±0.21 which was comparable to the standard drug Atorvastatin with values of total cholesterol as 163.79±2.21mg/dl, LDL-C as 105.98±2.32mg/dl, VLDL-C as 28.78±0.35mg/dl, HDL-C as 29.03±1.18mg/dl, atherogenic index as 5.68±0.22 respectively. These findings support the use of the extract as an adjuvant with existing therapy for treatment of hyperlipidemia.

KEYWORDS

Hyperlipidemia, Atorvastatin, Eugenia caryophyllus, Atherogenic index.

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INTRODUCTION

Hyperlipidemia is a metabolic disorder specifically characterized by increased concentration of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides with a concomitant decrease in the concentration of high density lipoproteins (HDL-C) in the blood circulation. Recent studies emphasized that serum cholesterol, both total and lipoprotein fractions, are associated with mid- and late-life depression. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis associated conditions like coronary heart disease (CHD), ischaemic cerebrovascular disease and peripheral vascular disease, hence it serves as one of the leading causes of death in the developed countries and is on the rise in developing countries like India. World Health Organization (WHO) reports that high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year. Therefore therapists consider the treatment of hyperlipidemia to be one of the major approaches towards decelerating the atherogenic process, thus reducing the risk of developing ischaemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease.

*Eugenia caryophyllus* is a small ever-green tree belonging to family *Myrtaceae* (subfamily: *Myrtoideae* and tribe: *Syzgieae*) and is scientifically known as *Syzygium aromaticum* (L.) Merr. and L. M. Perry. Dried flower buds of *Eugenia caryophyllus* are used as spice. Recently we have shown in our previous studies that the aqueous ethanolic extract of flower buds of *Eugenia caryophyllus* showed the presence of chemical compound confirmed to be Gossypetin 7-O rhamnopyranoside (Rhodiolgin) which was further characterized for its bioactive properties. The essential oil obtained from the buds of *Eugenia caryophyllus* L. is widely known for its medicinal properties. Chemical analysis has identified the major constituents as eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone. Its oil possesses anti-oxidant properties. It is significant in dentistry being used as a topical application to relieve pain and to promote healing. It is also a crucial component for fragrance and flavouring industries. The essential oil has shown to have anti-microbial activity, anti-fungal properties against dermatophytes. Futhermore, it is found to have antimutagenic, anti-inflammatory, antiulcerogenic, antithrombotic and antiparasitic and an anti-convulsant activity. Pharmacological studies with *Eugenia caryophyllus* extract have also exhibited anti-stress activity, analgesic activities and found to be more potent than aspirin in inhibiting platelet aggregation. Recently a study was conducted in our lab demonstrating anti-hyperlipidemic potential of *Eugenia caryophyllus* using animal model of Triton induced hyperlipidemia.

Currently available hypolipidaemic drugs have been associated with a number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. Therefore it is a need of the day to search other materials from natural sources which besides being less toxic and less expensive, provide better safety and efficacy on a long term usage. As herbal medicines are less damaging than synthetic drugs, they have better compatability, thus improving patient compliance on long term use. Polyherbal capsules consisting of various anti-lipidemic plant parts were reported to be crucial for the treatment and management of dyslipidemia. The present study was undertaken to investigate lipid lowering activity of the aqueous ethanolic extract of flower buds of *Eugenia caryophyllus* using high fat diet induced hyperlipidemia model.
Plant material

The flower buds of *Eugenia caryophyllus* were collected from an authorized vendor, ‘Global Herbs’ and authenticated by Professor Mohammed Ali, Department of Pharmacognosy, Faculty of Pharmacy, Jamia Hamdard, New Delhi. A voucher specimen coded PRL/JH/11/01 was deposited in the Jamia Hamdard for future reference.

Atherogenic diet and Chemicals

‘Experimental diet was made by mixing wheat powder (67.5g), Corn powder(62.5g), Barley powder (37.5g), Milk powder(37.5g), Animal fat(25g), Calcium chloride(2.5g), Salt(2.5g), Coconut oil(10ml), Vanaspati (10ml), Cholic acid(2g), Cholesterol(2g), Sugar(20g) and Vit.B12(1 tablet). The wet dough was dried at room temperature and rolled into small balls before feeding the animals. Atorvastatin was obtained from Alkem Research Centre, Navi Mumbai. All other chemicals were of analytical grade. Diagnostic test kits for measuring total cholesterol, Triglycerides, HDL-C, Serum glutamate oxaloacetate Transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin, glucose, uric acid and creatinine in serum were purchased from registered dealers.

Preparation of Plant extract

The flower buds of *Eugenia caryophyllus* were dried under shade at room temperature and reduced to coarse powder. The powdered material was subjected to qualitative tests for the identification of various phytoconstituents like alkaloids, flavonoids, phenols, phytosterols etc. 250g of powder was subjected to cold maceration with 500ml of 70% ethanol for 7 days at room temperature with in between stirring & shaking. After 7 days, it was filtered through Whatman filter paper 1 and the filtrate was then concentrated on water bath to obtain a dark brownish residue. The percentage yield obtained was 25%. The aqueous ethanolic extract i.e hydro-alcoholic extract of *Eugenia caryophyllus* will be called as EEC. The extract was suspended in 1% gum acacia for oral administration.

Experimental Animals

Wistar albino adult rats weighing 120-150g of either sex were obtained from the animal house, KIET School of Pharmacy, Ghaziabad, India. The animals were grouped and housed in polycrylic cages (38x 23 x 10 cm) with not more than five animals per cage and maintained under standard laboratory conditions (temperature 25+2°C), relative humidity (50% ± 5%) and 12 h light and dark cycle. They were allowed free access to standard pellet rat chow diet (Amrut feeds, Pranav Agro Industries Ltd., New Delhi, India) and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment.

The present study was approved by Institutional Animal Ethical Committee (IAEC) of KIET School of Pharmacy, Ghaziabad constituted under CPCSEA (IAEC/KSOP/02/2013-2014 dtd 18.10.13)
Diet induced hyperlipidemic model

Hyperlipidemia was induced by oral feeding of high fat diet for six weeks. The rats were then administered plant extracts suspended in 1% gum acacia at the doses of 200mg/kg and 400mg/kg body weight once daily in the morning through gastric intubation for another six weeks. During these days, the animals received high fat diet. The control group was however provided with normal pellet chow (Amrut feeds, India) and water ad libitum.

Experimental Design

Animals were divided into five groups of six animals each.

- **Group I** – Normal diet + Water
- **Group II** – High fat diet + Water
- **Group III** – High fat diet + EEC (200mg/kg body weight p.o.)
- **Group IV** – High fat diet + EEC (400mg/kg body weight p.o.)
- **Group V** – High fat diet + Atorvastatin (10mg/kg body weight p.o.)

The body weight was taken on first day of induction of hyperlipidemia and then on weekly basis before the treatment and after the treatment.

Collection of blood

On the last day of experiment, the blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected blood samples were centrifuged for 10 minutes at 2000 rpm and serum samples so collected were used for various biochemical experiments. The animals were then sacrificed and the liver tissue was collected.

Biochemical analysis in serum

The serum was assayed for total cholesterol (TC) and high-density lipoprotein (HDL-C) by CHOD-POD method, triglycerides.

LDL-C and VLDL-C were calculated using Friedwald formula.

\[
\text{VLDL} = \frac{\text{Triglycerides}}{5} \quad \text{VLDL} = \text{TC} - (\text{HDL} + \text{VLDL})
\]

Atherogenic index was calculated as A.I= Total Cholesterol/HDL-C.

The serum was assayed for glucose by GOD-POD method to assess its effect on liver and pancreas. SGOT and SGPT were measured by modified IFCC method. ALP was measured by modified Kind & King’s method. Bilirubin was assessed by modified Jendrassik and Grof’s method. Kidney profile viz. uric acid was measured by modified Trinder’s method and creatinine levels were estimated using Jaffé’s method in order to evaluate the effect of high fat diet and to investigate the effect of the plant extract on the kidneys.
Biochemical analysis of liver

In chilled normal saline, excised livers were perfused to remove all the blood cells. Then they were cut down into small pieces, placed in 0.1M phosphate buffer (pH 7.4), and homogenized using remi homogenizer to obtain 20% homogenate. The homogenate thus obtained was centrifuged at 3000 rpm for 15 min and the supernatant was collected in an Eppendorf tube. The supernatant was used for the study of lipid profile parameters like triglycerides, total cholesterol, HDL-C, LDL-C, VLDL-C and atherogenic index using methods described as earlier.

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis was carried out by using ANOVA followed by Dunnet’s multiple comparison tests using Graph pad PRISM software version 4.03 (Graph Pad Software Inc. San Diego, California, USA). P values < 0.05 were considered as statistically significant.

RESULTS

As earlier demonstrated in triton induced model, *Eugenia caryophyllus* was found to be non–toxic up to the dose of 2000mg/kg body weight and did not cause any death of the tested animals. On basis of the acute toxicity studies, the doses 1/10th i.e. 200mg and 1/20th i.e 400mg/kg were selected.

Effect of EEC on body weight of the animal

Table 1 shows the effect of extract obtained from flower buds of *Eugenia caryophyllus* (EEC) on body weight. It was seen that the high fat diet produced a significant increase in body weight by the end of sixth week. The extract when given along with high fat diet prevented increase in body weight.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (Normal Control)</td>
<td>126.1±1.7</td>
<td>127.0±1.6</td>
<td>128.5±1.4</td>
<td>130.3±1.3</td>
<td>132.1±1.7</td>
<td>133.8±1.9</td>
<td>135±1.7</td>
</tr>
<tr>
<td>Group II HFD ie. hyperlipidemic control</td>
<td>176.8±5.1</td>
<td>180.8±5.2a</td>
<td>183.7±5.1a</td>
<td>187.3±5.4a</td>
<td>191.1±5.8a</td>
<td>195.3±6.2a</td>
<td>199±6.3a</td>
</tr>
<tr>
<td>Group III (HFD+EEC 200mg/kg)</td>
<td>170.5±1.9</td>
<td>173.1±1.7</td>
<td>173.5±1.3</td>
<td>173.3±1.4b</td>
<td>174.5±1.2b</td>
<td>176.0±1.3b</td>
<td>176.5±1.0b</td>
</tr>
<tr>
<td>Group IV (HFD+EEC 400mg/kg)</td>
<td>176.8±6.8</td>
<td>178.6±6.9</td>
<td>179.8±7.1</td>
<td>179.1±6.9</td>
<td>179.8±7.5</td>
<td>181.5±7.0</td>
<td>182±7.7</td>
</tr>
<tr>
<td>Group V (HFD+Atorvastatin 10mg/kg)</td>
<td>161±4.8</td>
<td>163±4.7</td>
<td>165±4.9b</td>
<td>166.1±4.3b</td>
<td>166.1±4.5b</td>
<td>166±4.4b</td>
<td>164.3±3.9b</td>
</tr>
</tbody>
</table>

HFD : high fat diet, EEC : aqueous ethanolic extract of *Eugenia caryophyllus*
Values are expressed as mean μ±SEM(n=6). \(^\text{a}\)P<0.05 vs Group I and \(^\text{b}\)P<0.05 vs Group II, using one way ANOVA followed by Dunnet’s test.

**Effect of EEC on lipid profile and glucose in serum**

Table 2 shows the effect of extract obtained from *Eugenia caryophyllus* on lipid profile and glucose. In the present study it was found that there was an extremely significant (P<0.05) increase in serum total cholesterol, triglycerides, low density lipoprotein (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and atherogenic index in high fat treated group as compared to normal control group. All the treated groups showed an extremely significant (P<0.05) decrease in serum total cholesterol, triglycerides, LDL-C, VLDL-C and atherogenic index. In comparison to 200mg /kg, the dose 400mg/ kg possessed better antihyperlipidemic property. There was an extremely significant (P<0.05) decrease in serum HDL cholesterol in high fat treated group as compared to normal control. Atorvastatin 10mg/kg, the extract at doses 200mg/kg and 400 mg/ kg showed an extremely significant increase in HDL cholesterol as compared to hyperlipidemic control group. Among all 400 mg/ kg was found to be most protective group. There was an extremely significant (P<0.05) increase in serum glucose level of hyperlipidemic control group as compared to normal control group. 200mg/kg and extract at 400 mg/ kg showed an extremely significant decrease in serum blood glucose level as compared to hyperlipidemic control group. The response was seen similar to that of standard drug Atorvastatin at the dose of 10mg /kg body weight.

**Table 2: Effect of aqueous ethanolic extract of Eugenia caryophyllus on total cholesterol, HDL-C, LDL-C, VLDL-C, triglycerides, atherogenic index and glucose in serum of Control and Experimental rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Atherogenic index</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I  (Normal Control)</td>
<td>76.9 ± 1.7</td>
<td>27.4±1.0</td>
<td>32.4±1.8</td>
<td>17.1±0.2</td>
<td>85.5± 0.9</td>
<td>2.8±0.1</td>
<td>107.4±1.6</td>
</tr>
<tr>
<td>Group II (HFD ie. hyperlipidemic control)</td>
<td>247.5±1.5 (^\text{a})</td>
<td>14.9±0.9 (^\text{a})</td>
<td>191.4±2.0 (^\text{a})</td>
<td>41.1±0.5 (^\text{a})</td>
<td>205.6±2.6 (^\text{a})</td>
<td>16.8±0.9 (^\text{a})</td>
<td>152.3±1.2 (^\text{a})</td>
</tr>
<tr>
<td>Group III (HFD+EEC200mg/kg)</td>
<td>155.4±1.4 (^\text{b})</td>
<td>26.67±0.9 (^\text{b})</td>
<td>97.1±2.0 (^\text{b})</td>
<td>31.5±0.5 (^\text{b})</td>
<td>157.9±2.5 (^\text{b})</td>
<td>5.8±0.2 (^\text{b})</td>
<td>120.5±1.5 (^\text{b})</td>
</tr>
<tr>
<td>Group IV (HFD+EEC 400mg/kg)</td>
<td>145.8±1.1 (^\text{bc})</td>
<td>29.4±1.8 (^\text{b})</td>
<td>87.8±1.7 (^\text{bc})</td>
<td>28.5±0.3 (^\text{bc})</td>
<td>142.6±1.5 (^\text{bc})</td>
<td>4.9±0.2 (^\text{b})</td>
<td>116.7±1.2 (^\text{b})</td>
</tr>
<tr>
<td>Group V  (HFD+Atorvastatin 10mg/kg)</td>
<td>163.7±2.2 (^\text{b})</td>
<td>29.0±1.1 (^\text{b})</td>
<td>105.9±2.3 (^\text{b})</td>
<td>28.7±0.3 (^\text{b})</td>
<td>143.9±1.7 (^\text{b})</td>
<td>5.6±0.2 (^\text{b})</td>
<td>121.5±1.0 (^\text{b})</td>
</tr>
</tbody>
</table>

HFD : high fat diet, EEC : aqueous ethanolic extract of *Eugenia carophyllus*

Values are expressed as mean μ±SEM(n=6). \(^\text{a}\)P<0.05 vs Group I and \(^\text{b}\)P<0.05 vs Group II, \(^\text{c}\)P<0.05 vs Group III using one way ANOVA followed by Dunnet’s test.

**Effect of EEC on ALP, SGOT, SGPT, bilirubin, creatinine and uric acid levels in serum**
Table 3 shows the effect of extract from *Eugenia caryophyllus* on liver profile in hyperlipidemic rats. High fat diet produced a significant increase in ALP, SGOT, SGPT and bilirubin levels. However the aqueous alcholic extract from *Eugenia caryophyllus* restored the parameters to normal levels. A significant increase in the level of serum creatinine and uric acid was also observed in high fat diet fed rats, however the extract lowered it back to normal levels.

**Table 3:** Effect of aqueous ethanolic extract of *Eugenia caryophyllus* (EEC) on ALP, SGOT and SGPT, Creatinine and uric acid in serum of Control and Experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (U/L)</th>
<th>SGPT (U/L)</th>
<th>SGOT (U/L)</th>
<th>Bilirubin (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal Control)</td>
<td>73.8±2.1</td>
<td>39.4±1.3</td>
<td>49.7±1.0</td>
<td>0.6±0.0</td>
<td>0.7±0.0</td>
<td>2.8±0.0</td>
</tr>
<tr>
<td>Group II (HFD i.e. hyperlipidemic control)</td>
<td>310.0±1.9</td>
<td>119.9±1.2</td>
<td>133.7±1.1</td>
<td>0.7±0.0</td>
<td>1.9±0.0</td>
<td>5.6±0.0</td>
</tr>
<tr>
<td>Group III (HFD+EEC200mg/kg)</td>
<td>189.1±2.6</td>
<td>48.6±1.6</td>
<td>70.6±1.3</td>
<td>0.6±0.0</td>
<td>1.0±0.0</td>
<td>3.2±0.0</td>
</tr>
<tr>
<td>Group IV (HFD+EEC 400mg/kg)</td>
<td>106.0±1.7</td>
<td>36.1±1.5</td>
<td>63.8±1.7</td>
<td>0.3±0.0</td>
<td>0.8±0.0</td>
<td>3.0±0.0</td>
</tr>
<tr>
<td>Group V (HFD+ Atorvastatin 10mg/kg)</td>
<td>148.9±1.5</td>
<td>53.6±1.4</td>
<td>62.2±1.2</td>
<td>0.2±0.0</td>
<td>1.0±0.0</td>
<td>3.7±0.0</td>
</tr>
</tbody>
</table>

HFD : high fat diet, EEC : aqueous ethanolic extract of *Eugenia caryophyllus*

Values are expressed as mean μ±SEM(n=6). *P<0.05 vs Group I and †P<0.05 vs Group II, ‡P<0.05 vs Group III using one way ANOVA followed by Dunnet’s test.

**Effect of EEC on total cholesterol, LDL-C, VLDL-C, triglycerides, atherogenic index in liver tissue in Control and Experimental rats**

Table 4 below shows the effect of extract from *Eugenia caryophyllus* on lipid profile in the liver tissue of hyperlipidemic rats. It was seen that high fat diet also produced a significant increase in total cholesterol, LDL-C, VLDL-C, triglycerides and atherogenic index in the liver tissue. However treatment with the aqueous ethanolic extracts of *Eugenia caryophyllus* significantly lowered down the levels. It was also observed that high fat diet decreased the levels of HDL-C; however the extract not only prevented further decrease but also increased HDL levels.
Table 4: Effect of aqueous ethanolic extract of *Eugenia caryophyllus* on the lipid profile in liver of Control & Experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglyceride (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>Atherogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal Control)</td>
<td>8.6±0.3</td>
<td>13.5±0.6</td>
<td>4.6±0.1</td>
<td>7.1±0.6</td>
<td>1.7±0.1</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>Group II (HFD ie. hyperlipidemic control)</td>
<td>20.8±0.5(^a)</td>
<td>43.5±1.7(^a)</td>
<td>2.5±0.1(^a)</td>
<td>36.8±1.8(^a)</td>
<td>4.1±0.1(^a)</td>
<td>17.6±1.4(^a)</td>
</tr>
<tr>
<td>Group III (HFD+EEC 200mg/kg)</td>
<td>16.0±0.7(^b)</td>
<td>27.3±0.8(^b)</td>
<td>4.5±0.1(^b)</td>
<td>19.6±0.8(^b)</td>
<td>3.2±0.1(^b)</td>
<td>6.0±0.1(^b)</td>
</tr>
<tr>
<td>Group IV (HFD+EEC 400mg/kg)</td>
<td>14.4±0.5(^b)</td>
<td>25.6±1.0(^b)</td>
<td>4.9±0.2(^b)</td>
<td>17.7±1.0(^b)</td>
<td>2.9±0.1(^b)</td>
<td>5.2±0.3(^b)</td>
</tr>
<tr>
<td>Group V (HFD+ Atorvastatin (10mg/kg))</td>
<td>14.6±0.6(^b)</td>
<td>28.7±0.9(^b)</td>
<td>4.8±0.1(^b)</td>
<td>20.9±1.0(^b)</td>
<td>2.9±0.1(^b)</td>
<td>5.9±0.3(^b)</td>
</tr>
</tbody>
</table>

HFD : high fat diet, EEC : aqueous ethanolic extract of *Eugenia caryophyllus*

Values are expressed as mean μ±SEM(n=6). \(^a\)P<0.05 vs Group I and \(^b\)P<0.05 vs Group II using one way ANOVA followed by Dunnet’s test.

**DISCUSSION**

From time immemorial, plants have served as the best source of medicine for the treatment of various disorders with no or negligible side effects. Hyperlipidemia has been associated with heart disease, which is the leading cause of death in the world. Several studies revealed that an increase in HDL cholesterol and a decrease in total cholesterol, LDL-C, triglycerides is associated with a decrease in the risk of ischaemic heart disease\(^28\). The mediating effects on the associations of serum lipids with depressive symptoms have been reported to be statistically significant for ischemic heart disease and stroke\(^29\). Most of the currently available marketed antihyperlipidemic drugs are causing significant reduction in both TC and HDL-C levels and are also known to cause innumerable side effects therefore the significance of herbal drugs in the treatment of hyperlipidemia has received considerable attention in recent years. The lowering of the levels of harmful lipids to satisfactory values has been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The present study elucidated the role of *Eugenia caryophyllus* extract during hyperlipidemia induced by high fat diet in Wistar rats. It was observed that there was a marked increase in the levels of serum total cholesterol, triglycerides, LDL-C, VLDL-C and a decrease in the level of good cholesterol carrier HDL in animals treated with high fat diet. In the present study, treatment with the aqueous ethanolic extract of *Eugenia caryophyllus* at the doses of 200mg/kg and 400mg/kg reduced the levels of serum total cholesterol, triglycerides, VLDL-C, LDL-C and raised good cholesterol carrier HDL as compared to hyperlipidemic control group.
High fat induced model has been used as a screening method for hyperlipidemic agents and also used for elucidating lipid metabolism. In the present study those ingredients of high fat diet are chosen which forms a part of daily food. Diet containing saturated fatty acids increases the activity of HMGCoA reductase, the rate determining enzyme in cholesterol biosynthesis\(^3\). This may be due to higher availability of acetyl CoA which stimulated the cholesterologenic rate. Moreover this could be associated with down regulation of LDL receptors by cholesterol and saturated fatty acids in diet which could explain the elevation of serum LDL-C levels\(^3\). LDL transports cholesterol from liver to other peripheral tissues. The reduction of total cholesterol by aqueous ethanolic extract of *Eugenia caryophyllus* was associated with a decrease of its LDL fraction in serum and liver which is a target of several antihyperlipidemic drugs. These results could be due to rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids or reduction of lipid absorption in intestine\(^3\).

The reduction in the triglycerides level may be due to an increase in the activity of endothelial bound lipoprotein lipase that hydrolyzes the triglycerides into fatty acids or could be inhibition of lipolysis so that fatty acids do not get converted into triglycerides\(^3\).

HDL-C works as a cardioprotective protein since an independent inverse relationship between blood HDL levels and cardiovascular risk incidence has been reported\(^3\). HDL increase was observed in the present study when treated with aqueous ethanolic extract of *Eugenia caryophyllus*.

Since the atherogenic index of plasma (AIP) predicts the presence of small, dense, and highly atherogenic low density lipoprotein (LDL) and high density lipoprotein (HDL) particles therefore it serves as a crucial tool for screening dyslipidemia\(^3\). In the present study aqueous ethanolic plant extract was found to reduce the atherogenic index significantly.

Earlier studies conducted in our lab have confirmed the presence of moderate quantity of flavanoids and polyphenols in the aqueous alcoholic extract of *Eugenia caryophyllus* (unpublished data). It is known that plant flavanoids and polyphenols have exhibited a variety of pharmacological activities including the antiatherogenic effect\(^3\). The results strongly suggest that the lipid lowering activity of the extract could be attributed to the presence of flavanoid phytoconstituents.

The results of the present study also indicated that animals fed on high fat diet has increased bilirubin, SGOT, SGPT and ALP levels as compared to normal control. Rats treated with extracts of aqueous alcoholic extract of *Eugenia caryophyllus* caused significant decrease in the levels of SGOT, SGPT and ALP activities at doses of 200mg/kg and 400mg/kg respectively.

There was also a significant increase in the level of serum creatinine and uric acid in high fat diet fed rats which were restored back to normal levels by administration of the aqueous alcoholic extract of *Eugenia caryophyllus*.

**CONCLUSION**

The findings of the present study revealed that *Eugenia caryophyllus* in both low (200mg/kg) and high (400mg/kg) doses showed antihyperlipidemic activity against high fat diet induced hyperlipidemia. Among all the treated groups, *Eugenia caryophyllus* extract at the dose of 400mg/kg had shown better protection. The anti-hyperlipidemic activity of the plant could be
attributed to the presence of flavanoids in them; In retrospect to the significant role played by the alcoholic extract of *Eugenia caryophyllus* as antihyperlipidemic substance and thereby preventing the chances of fatal cardiovascular disorders, further research revealing that its mechanism of action at molecular level would be crucial for treating various disorders associated with hyperlipidemia.

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